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Short communication

Simultaneous determination of dopamine and serotonin in the presence of ascorbic acid and uric acid at poly(*o*-phenylenediamine) modified electrode

T. SELVARAJU and R. RAMARAJ*
School of Chemistry, Madurai Kamaraj University, Madurai – 625 021, India
*(*author for correspondence, fax: +91 452 2459139, e-mail: ramarajr@yahoo.com)*

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1. Introduction

Dopamine (DA) is an important neurotransmitter molecule of catecholamines and its deficiency leads to brain disorders such as Parkinson's disease and schizophrenia [1–3]. Thus detecting and determining the concentrations of dopamine and their metabolites in the presence of interfering species is an important goal in electrochemical analysis. Much attention has been given to the design and development of novel materials coated on electrode surfaces with improved molecular recognition capabilities [4, 5]. Different types of electrode materials are tailored to identify and measure the neurotransmitter molecules [6–10]. Measurements at bare glassy carbon electrode are complicated due to coexistence of high concentration of ascorbic acid (AA) and other related species, which are oxidized at the same potential region [8]. Uric acid (UA) also attenuates the detection of neuromolecules such as dopamine (DA) and serotonin (5-hydroxytryptamine (5-HT)) though 5-HT undergoes oxidation at more positive potential [10]. Several types of electrode modifications are reported in the literature to detect dopamine by electrochemical methods such as surface coating [6], carbon paste electrodes [10], self assembled monolayers [11]. However, each type of modification has its own advantages and limitations. Simultaneous measurement of dopamine and serotonin in *in vivo* studies has been mainly unsuccessful due to several interfering species existing in the living systems. A few studies are reported for the simultaneous detection of DA and 5-HT [10, 12].

Detection of DA and 5-HT in the presence of AA and UA using conducting polymers such as poly(*o*-phenylenediamine) (PPD) prepared by electropolymerization of 1,2-diaminobenzene has not been exploited. Recently, Mo and Ogorevc [13] reported the electropolymerization of 1,2-diaminobenzene (*o*-phenylenediamine) in the presence of sodium dodecyl sulfate on carbon fibre microelectrode (represented as PPD-SDS), and the overoxidation of PPD coated microelectrode at 2.2 V was found to be applicable for the simultaneous determination of both cationic DA and anionic AA. The overoxidation of PPD coated carbon fibre microelectrode may lead to the formation of carbonyl groups and carboxylic groups on the electrode surface due to electrode surface oxidation [14]. Malitesta and coworkers prepared PPD film coated glassy carbon electrode by electropolymerization of a mixture containing 1,2-diaminobenzene and glucose oxidase enzyme and used as glucose sensor. Their preliminary experiments [15] also showed that the electrochemistry of anionic AA, UA and DOPAC and cationic DA and NE (norepinephrine) molecules were nearly completely suppressed and showed a response similar to other nonconducting films. The PPD modified electrode prepared by electropolymerization of a mixture containing 1,2-diaminobenzene and a nonionic surfactant such as octaethylene glycol monohexadecyl ether using 0.1 mol dm^{-3} KCl (pH 7) as supporting electrolyte showed permselectivity, whereas in the absence of nonionic surfactant, the PPD film coated electrode does not show permselectivity towards positively charged redox couples [16]. In this communication, we report the simultaneous detection of DA and 5-HT in the presence of both AA and UA at the modified electrode prepared by electropolymerization of 1,2-diaminobenzene in highly acidic condition (pH < 1). The PPD modified electrode selectively oxidizes DA or 5-HT in a mixture containing DA or 5-HT, AA and UA in 0.1 mol dm^{-3} phosphate buffer pH 7.1.

2. Experimental details

Dopamine (3-hydroxytyramine), serotonin (5-hydroxytryptamine) and uric acid were obtained from Sigma and used as received. Ascorbic acid (BDH Merck) was used without further purification. Freshly prepared DA and 5-HT solutions were used for electrochemical analysis. Electrochemical experiments were performed in a conventional two-compartment three-electrode cell at room temperature (25 °C). The reference electrode was a saturated calomel electrode (SCE). The electrolyte solution was purged with pure nitrogen for 20 min prior to each experiment. Glassy carbon (GC) electrode was used as working electrode (0.07 cm^2) and platinum foil as counter electrode. The GC electrode was twice polished using alumina powder (200–300 mesh) followed

by sonication in double distilled water for 5 min. 1,2-Diaminobenzene was used after thrice recrystallized from water. 1,2-Diaminobenzene was electropolymerized on GC electrode in 0.5 mol dm^{-3} sulfuric acid by cycling between −0.2 and 1.1 V at a scan rate of 50 mV s^{-1} (25 cycles). Experiments were carried out in 0.1 mol dm^{-3} phosphate buffer solution at pH 7.1. The solutions were prepared in doubly distilled water. The electrochemical experiments were carried out with a 283 potentiostat/galvanostat controlled by the M270 software (EG&G, Princeton, NJ).

3. Results and discussion

The continuous cyclic voltammograms recorded using GC electrode dipped in a mixture containing 0.01 mol dm^{-3} 1,2-diaminobenzene and 0.5 mol dm^{-3} H_2SO_4 under deaerated conditions showed the oxidation of monomer 1,2-diaminobenzene at 1.0 V in the first cycle (curve not shown). In the subsequent cycles, the oxidation current was decreased at 1.0 V with a simultaneous appearance of a redox wave at 0.02 V due to the formation of PPD. This clearly shows the oxidation of the monomer 1,2-diaminobenzene and the formation of PPD film on the GC electrode. (represent as GC/PPD). It is reported that the PPD polymerization process exhibits a nearly Nernstian response to change in pH [16–18]; however, the detailed characterization is difficult. Figure 1(a) shows the cyclic voltammogram of GC/PPD electrode in deaerated 0.1 mol dm^{-3} phosphate buffer pH 7.1. The GC/PPD electrode does not show any redox behaviour in 0.1 mol dm^{-3} phosphate buffer (Figure 1). Figure 1(b) shows the cyclic voltammogram recorded for a mixture containing 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA in 0.1 mol dm^{-3} phosphate buffer. Figure 1(b) indicates that the PPD film on the electrode surface was completely blocked the oxidation of both AA and UA. Therefore, both AA and UA remains unoxidized at the PPD modified electrode surface. Figure 1(c) shows a typical cyclic voltammogram recorded at GC/PPD electrode dipped in deaerated 0.1 mol dm^{-3} phosphate buffer containing a mixture of 1×10^{-4} mol dm^{-3} DA, 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA. Here we observed both oxidation and reduction peaks. The redox potential of dopamine was correlated with the redox peaks (Figure 1(c)) [8]. Therefore we confirmed that dopamine alone selectively oxidized in the presence of high concentration of ascorbic acid and uric acid. Figure 2 shows the linear plot obtained for [DA] against the anodic peak current (i_{pa}) at the scan rate 50 mV s^{-1}.

Similar electrochemical behaviour was also observed in the differential pulse voltammetric studies for the determination of dopamine at GC/PPD electrode. Figure 3(a) shows the differential pulse voltammogram (DPV) of GC/PPD electrode in deaerated 0.1 mol dm^{-3} phosphate buffer and the PPD film does not show oxidation peak as discussed earlier. Figure 3(b) shows the DPV recorded for a mixture of 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA in 0.1 mol dm^{-3} phosphate buffer. The DPV (Figure 3(b)) indicates that the PPD film attenuates the oxidation of both AA and UA at the GC/PPD modified electrode. Figure 3(c) and (d) shows the DPVs obtained for 2×10^{-5} mol dm^{-3} DA at the GC/PPD electrode in the absence (Figure 3(c)) and presence (Figure 3(d)) of 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA, respectively, in 0.1 mol dm^{-3} phosphate buffer. It exhibits that the electrochemistry of DA was not blocked at the PPD film coated electrode (as shown in Figure 1(c)). The results indicate that the PPD film formed in 0.5 mol dm^{-3} H_2SO_4 on the GC electrode shows permselectivity, allowing the positively charged molecules to interact at the PPD coated electrode while rejecting the negatively charged molecules. The polymer modified electrode shows an excellent electrooxidation of DA with decrease in their

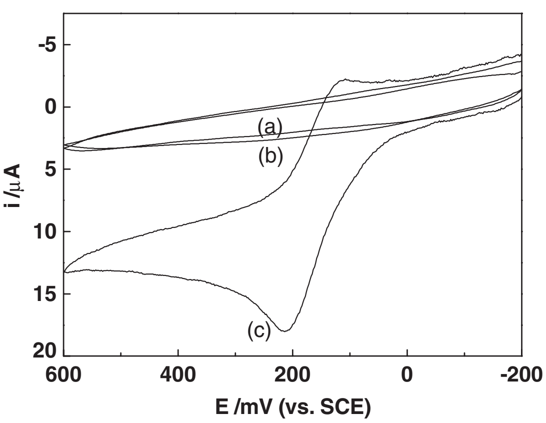

Fig. 1. Cyclic voltammograms of poly(*o*-phenylenediamine) modified electrode dipped in 0.1 mol dm^{-3} aqueous phosphate buffer pH 7.1: (a) in the absence of DA, AA and UA; (b) in the presence 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA; and (c) in the presence of mixture containing 1×10^{-4} mol dm^{-3} DA, 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA. Scan rate 50 mV s^{-1}.

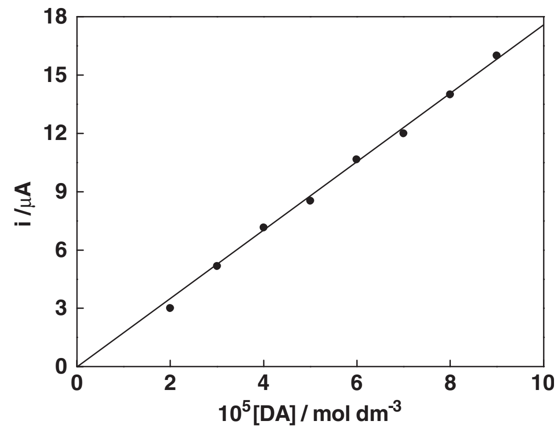

Fig. 2. Linear plot of the anodic peak current (i_{pa}) against [DA] for GC/PPD electrode in 0.1 mol dm^{-3} aqueous phosphate buffer pH 7.1. Scan rate 50 mV s^{-1}.

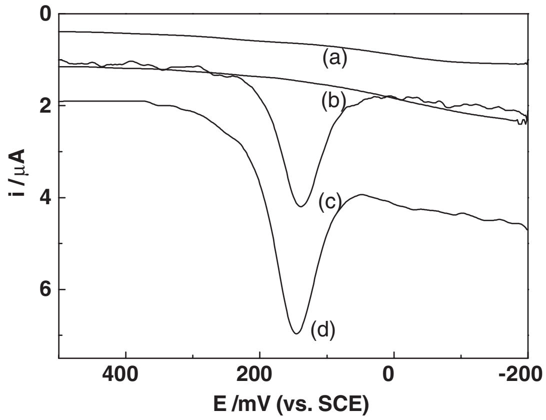

Fig. 3. Anodic differential pulse voltammograms (DPV) of poly(*o*-phenylenediamine) modified electrode dipped in 0.1 mol dm^{-3} aqueous phosphate buffer pH 7.1: (a) in the absence of DA, AA and UA; (b) in the presence of 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA; (c) in the presence of 2×10^{-5} mol dm^{-3} DA; and (d) in a mixture containing 2×10^{-5} mol dm^{-3} DA, 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA. Scan rate 5 mV s^{-1}.

overpotential, which is about 100 mV lower than that at the bare electrode. The DPV results shows that the detection of DA is possible in 50-fold excess of AA and UA and it is established by using the PPD modified electrode. The interaction of positively charged DA at PPD electrode was further supported by recording the cyclic voltammogram of positively charged $[Ru(NH_3)_6]^{3+}$ and negatively charged $[Fe(CN)_6]^{3-}$ at the same GC/PPD film coated electrode in 0.5 mol dm^{-3} KCl as supporting electrolyte. As expected, the electrochemistry of $[Ru(NH_3)_6]^{3+}$ was not blocked at the GC/PPD electrode, whereas the electrochemistry of $[Fe(CN)_6]^{3-}$ was blocked at the same electrode, but bare GC electrode gives good cyclic voltammetric response for the same $[Fe(CN)_6]^{3-}$ as shown in Figure 4.

The electrochemical behaviour observed for $[Ru(NH_3)_6]^{3+}$ and $[Fe(CN)_6]^{3-}$ at GC/PPD electrode in the present work is very similar to the earlier report [16]. In the present work, PPD film prepared by using 0.5 mol dm^{-3} H_2SO_4 as supporting electrolyte containing 0.01 mol dm^{-3} 1,2-diaminobenzene exhibit permselectivity towards cationic charged species. The PPD modified electrode prepared using 0.1 mol dm^{-3} H_2SO_4 or a mixture containing 0.1 mol dm^{-3} H_2SO_4 and 0.1 mol dm^{-3} Na_2SO_4 or 0.1 mol dm^{-3} $HClO_4$ and 0.1 mol dm^{-3} $NaClO_4$ as supporting electrolyte did not show permselectivity towards dopamine and other cationic species and acted as an insulator. Thus the anion repulsion and cation nonrepulsion behaviour of PPD film was achieved only when the PPD film was prepared using higher concentration of H_2SO_4. The permselectivity property of the PPD film was also achieved during the electropolymerization of 1,2-diaminobenzene using 1.0 mol dm^{-3} $HClO_4$ as supporting electrolyte. The cation nonrepulsion behaviour of the PPD film may be due to the incorporation of SO_4^{2-} or ClO_4^- species into the PPD film [16]. When the supporting electrolyte concentration was further increased from 0.5 mol dm^{-3} H_2SO_4 to 1.0 mol dm^{-3} during electropolymerization of 1,2-diaminobenzene, the electrochemical behaviour of the PPD modified electrode was not changed significantly.

We have also attempted the simultaneous determination of DA and 5-HT in the presence of AA and UA. The DPV shown in Figure 5(a) does not show the oxidation peak for a mixture containing 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA at GC/PPD electrode in deaerated 0.1 mol dm^{-3} phosphate buffer. When a mixture containing 2×10^{-5} mol dm^{-3} DA, 2×10^{-5} mol dm^{-3} 5-HT, 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA in 0.1 mol dm^{-3} phosphate buffer, the DPV (Figure 5(b)) showed two peaks at 160 and 280 mV corresponding to the oxidation of DA and 5-HT at GC/PPD modified electrode. As described previously the electrochemistry of AA and UA was completely suppressed at the GC/PPD electrode. Figure 5(b) clearly demonstrates the simultaneous determi-

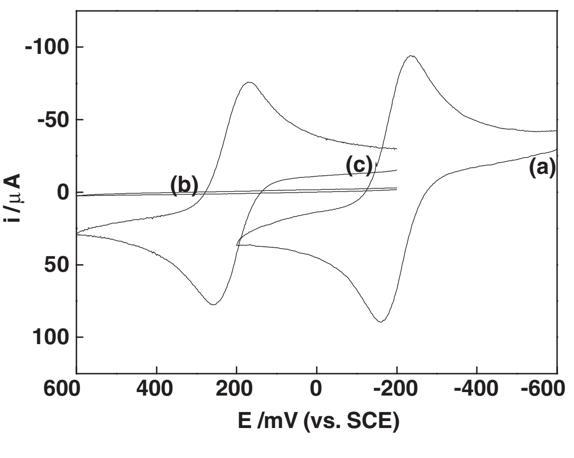

Fig. 4. Cyclic voltammograms of: (a) 5×10^{-3} mol dm^{-3} $[Ru(NH_3)_6]^{3+}$; (b) 5×10^{-3} mol dm^{-3} $[Fe(CN)_6]^{3-}$ at GC/PPD electrode; and (c) 5×10^{-3} mol dm^{-3} $[Fe(CN)_6]^{3-}$ at bare GC electrode in 0.5 mol dm^{-3} KCl (aq). Scan rate 50 mV s^{-1}.

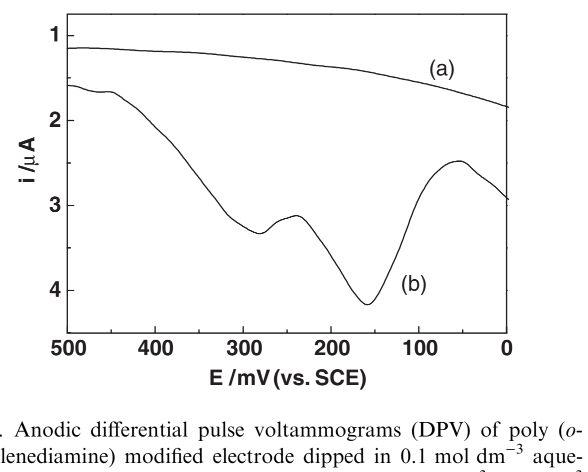

Fig. 5. Anodic differential pulse voltammograms (DPV) of poly (*o*-phenylenediamine) modified electrode dipped in 0.1 mol dm^{-3} aqueous phosphate buffer pH 7.1: (a) in the presence of 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA; and (b) in a mixture containing 2×10^{-5} mol dm^{-3} DA, 2×10^{-5} mol dm^{-3} 5-HT, 1×10^{-3} mol dm^{-3} AA and 1×10^{-3} mol dm^{-3} UA. Scan rate 5 mV s^{-1}.

nation of DA and 5-HT in the presence of AA and UA at GC/PPD electrode. Further, we found that the PPD modified electrode was stable for more than a week. The GC/PPD electrode kept in 0.1 mol dm^{-3} phosphate buffer showed only 5% decrease in the DA oxidation current after one week. The detection limit of DA was found to be 5×10^{-7} mol dm^{-3} at GC/PPD electrode.

The PPD coated GC electrode was prepared using 0.5 mol dm^{-3} H_2SO_4 as supporting electrolyte and the cyclic voltammetric behaviour obtained for GC/PPD electrode was very similar to a recent report [19]. As Elliott et al. [16] pointed out; the PPD film exhibits intrinsic negative charge possible through the incorporation of anions such as Cl^-, Br^-. The PPD film could be neutral, but will exhibit anion repulsion as a result of high electron density of aromatic rings and nitrogen lone pairs [16, 18]. In the present investigation, the PPD film prepared using higher concentration of supporting electrolyte shows the permselectivity towards the detection of DA and 5-HT in the presence of AA and UA. Thus the simultaneous determination of positively charged neurotransmitter molecules such as DA and 5-HT in the presence of negatively charged interferences such as AA and UA using the GC/PPD electrode by the electroanalytical techniques such as cyclic voltammetry and differential pulse voltammetry is possible. Further work is in progress.

4. Conclusion

The present study demonstrates the electrochemical polymerization of 1,2-diaminobenzene on glassy carbon electrode in acidic condition and its permselective behaviour towards electrooxidation and simultaneous determination of dopamine and serotonin in the presence of interferrents like ascorbic acid and uric acid. A 100 mV negative shift in the oxidation of DA is observed at the PPD modified electrode compared to the bare electrode.

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